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Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase

(transferase/excitatory neurotransmission/*N*-acetylaspartylglutamate/*N*-acetylated α-linked acidic dipeptidase/glutamate)

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ABSTRACT This report demonstrates that the investigational prostatic carcinoma marker known as the prostate-specific membrane antigen (PSM) possesses hydrolytic activity with the substrate and pharmacologic properties of the *N*-acetylated α-linked acidic dipeptidase (NAALADase). NAALADase is a membrane hydrolase that has been characterized in the mammalian nervous system on the basis of its catabolism of the neuropeptide *N*-acetylaspartylglutamate (NAAG) to yield glutamate and *N*-acetylaspartate and that has been hypothesized to influence glutamatergic signaling processes. The immunoscreening of a rat brain cDNA expression library with anti-NAALADase antisera identified a 1428-base partial cDNA that shares 86% sequence identity with 1428 bases of the human PSM cDNA (Israeli, R. S., Powell, C. T., Fair, W. R. & Heston, W. D. W. (1993) *Cancer Res.* *53*, 227–230). A cDNA containing the entire PSM open reading frame was subsequently isolated by reverse transcription-PCR from the PSM-positive prostate carcinoma cell line LNCaP. Transient transfection of this cDNA into two NAALADase-negative cell lines conferred NAAG-hydrolyzing activity that was inhibited by the NAALADase inhibitors quisqualic acid and β-NAAG. Thus we demonstrate a PSM-encoded function and identify a NAALADase-encoding cDNA. Northern analyses identify at least six transcripts that are variably expressed in NAALADase-positive but not in NAALADase-negative rat tissues and human cell lines; therefore, PSM and/or related molecular species appear to account for NAAG hydrolysis in the nervous system. These results also raise questions about the role of PSM in both normal and pathologic prostate epithelial-cell function.

Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and a diverse set of receptors activatable by glutamate and other glutamate-like molecules has been demonstrated to be involved in the processes of both rapid neuronal signaling and synaptic plasticity (1). Two important aspects of excitatory transmission still to be defined are the spectrum of endogenous ligands for the glutamate family of receptors and the specific mechanism(s) by which synaptic glutamate arises. It has been hypothesized that the acidic neuropeptide *N*-acetylaspartylglutamate (NAAG) might both serve as an endogenous receptor ligand and a glutamate presynaptic neurotransmitter stored within presynaptic nerve terminals. NAAG is localized to specific groups of neurons in brain (2), from which it is released in a calcium-dependent manner upon depolarization (3, 4). One puzzling aspect of the neurophysiology of NAAG has been the reports of excitatory (5, 6), inhibitory (7, 8), and no (5, 10) electrophysiologic effects observed upon its application to different neurons. These variable effects likely reflect the diverse actions of NAAG and its metabolites at members of the glutamate receptor family.

Intact NAAG is an agonist at a subpopulation of metabotropic glutamate receptors negatively coupled to adenylate cyclase (11) and antagonizes the effects of glutamate at the *N*-methyl-D-aspartate subtype of ionotropic receptors, where it is a weak agonist (8, 12). NAAG is also catabolized to glutamate and *N*-acetylaspartate in nervous tissue by the quisqualate-sensitive *N*-acetylated α-linked acidic dipeptidase (NAALADase) (13). The hydrolysis of NAAG by NAALADase to yield glutamate may thus be an important source of this neurotransmitter. Further, NAALADase is enriched in the synaptosomal fraction of brain (14), consistent with possible roles in both the termination of NAAG-mediated actions and the regulation of glutamate concentrations at neuronal synapses.

Supporting their relevance to glutamatergic signaling, NAAG and NAALADase have been implicated in disorders of the nervous system associated with the dysregulation of glutamatergic neurotransmission, such as amyotrophic lateral sclerosis (15, 16), schizophrenia (17), and seizure disorders (18, 19). The directions of the observed alterations in NAAG levels and NAALADase activity in these disorders correlate with the predicted changes in glutamate-receptor-mediated signaling.

One form of NAALADase has been purified to apparent homogeneity from rat brain (20). The purified enzyme is a glycoprotein with a native apparent molecular mass of ~94 kDa. Specific antisera raised against the purified glycoprotein demonstrate immunoreactivity that correlates with the distribution of NAALADase activity in rat brain, peripheral nerves, kidney, and sexual organs (20–22). The following report describes our use of these antisera to identify a human cell line cDNA that encodes a hydrolase activity with the substrate and pharmacologic properties of the NAALADase previously characterized in rat brain. The discovery of a NAALADase cDNA should prove to be a substantial advance toward understanding the neurobiologic function of NAAG and its likely role in the important process of glutamatergic signaling.

MATERIALS AND METHODS

Immunoscreening. The characteristics of the anti-NAALADase antisera that were used for immunoscreening have been reported (20). Immunoscreening of a λgt11 rat brain cDNA expression library obtained from Rachael Neve (McLean Hospital, Harvard Medical School, Boston) was conducted per Young and Davis (23) with a 1:100 dilution of primary antiserum.

Abbreviations: NAALADase, *N*-acetylated α-linked acidic dipeptidase; PSM, prostate-specific membrane antigen; NAAG, *N*-acetylaspartylglutamate; RT-PCR, reverse transcription-PCR; β-NAAG, *N*-acetyl-β-L-aspartyl-L-glutamate.

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Exhibit 1

50 immunopositive clones were subcloned into the *Eco*RI site of pBluescript SK− (Stratagene). Plasmid DNA was isolated using the Wizard DNA purification system (Promega).

DNA Sequencing and Analysis. Dideoxynucleotide sequencing reactions were done by using Sequenase kit 70770 (Amersham) or a *Pfu* (exo-) Cycle system (Stratagene) according to the manufacturer's instructions. Sequence analyses were conducted by using the Genetics Computer Group (Madison, WI) package, version 7.

Cell Lines. Tumor cell lines were obtained from the American Type Culture Collection. The DU145 and PC3 lines were grown in Dulbecco's minimal Eagle's medium/2 mM glutamine/10% fetal bovine serum. The LNCaP line was grown in RPMI 1640 medium/2 mM glutamine/nonessential amino acids/5% fetal bovine serum. All medium reagents were from GIBCO/BRL.

Enzyme Assays. Monolayer cultures (25-mm dish) of LNCaP, DU145, and PC3 cell lines were sonicated into 1.2 ml of ice-cold 50 mM Tris·HCl buffer (pH 7.4 at 37°C) containing 0.5% Triton X-100 by sonication. For comparative analyses, 100 μl of each cell-line lysate (20–100 μg of protein) was assayed for NAAG-hydrolyzing activity with or without the NAALADase inhibitors quisqualic acid (Research Biochemicals) (24, 25) and *N*-acetyl-β-L-aspartyl-L-glutamate (β-NAAG) (Bachem) (25). Thirty-minute radiometric assays were conducted in triplicate as described by Slusher *et al.* (20) and included cell-free blanks and rat brain tissue samples as negative and positive controls, respectively. For kinetic analysis of NAALADase in the LNCaP cell line, 500 ng of total protein was assayed at substrate concentrations of 15 nM–10 μM. Reported values are the means of three assays ± SEM.

Northern Blotting. Total RNA from adult male Sprague–Dawley rats (Taconic Farms) or cell lines was prepared by the method of Chirgwin *et al.* (26). RNA was separated by electrophoresis through a 1.2% agarose gel containing 3% formaldehyde, electrophoretically transferred to a nylon membrane, and hybridized to a random-primed ^{32}P-radiolabeled cDNA probe (specific activity = 1.5×10^9 dpm/μg) prepared using a Prime-It kit (Stratagene) at 42°C overnight. Final high-stringency washes were done with 0.1× standard saline citrate/0.1% SDS at either 65°C (rat RNA) or 55°C (human cell line RNA). The RNA was determined to be intact and evenly loaded by ethidium bromide staining and/or UV shadowing. Hybridization was detected by autoradiography using a Molecular Dynamic PhosphorImager.

Reverse Transcription (RT)–PCR Cloning of Prostate-Specific Membrane Antigen (PSM) cDNA. Reverse transcription reactions were conducted at 47–50°C for 2 hr using Superscript II reverse transcriptase (BRL) according to the manufacturer's recommended conditions with the addition of recombinant RNasin (Promega) at 40 units/25 μl and in some cases the addition of 3.33 mM dimethyl sulfoxide in the RNA denaturation step (equivalent to 2 mM final concentration in the transcription reaction). PCRs were done with native *Pfu* (Stratagene) or AmpliTaq (Perkin–Elmer) polymerase according to the supplier's recommendations using a GenAmp 480 thermal cycler (Perkin–Elmer). Thermal cycling parameters consisted of an initial denaturation step (94°C for 4 min) followed by 30–35 cycles of amplification (94°C for 1 min, 60–68°C for 1 min, 72°C for 3 min), and ending in a final extension step (72°C for 7 min). Primer sequences are as follows: primer 1, TGCAGGGCTGATAAGCGAG; primer 2, AGCCACGCCACGCTCTTG; primer 3, TCATCCAATTGGATACTATG; primer 4, TCTTTCTGAGTGACATAC. Primers were designed to amplify two cDNAs containing the 5′ and 3′ regions of the PSM coding sequence (bases 154–1605 and 1145–2565 of the Israeli *et al.* (27) clone. GenBank accession no. M99487) whose overlapping sequences would contain a native *Eco*RI restriction site (position 1572). The resultant cDNAs were joined using this *Eco*RI site and cloned into the *Bam*HI and *Xba*I sites of the mammalian expression plasmid pcDNA3 (Stratagene), resulting in plasmid PSMA3.

Transient Transfections. The 35-mm dishes of PC3 or DU145 cells were transfected with 5 μg of supercoiled plasmid DNA (prepared by the method of Radloff *et al.* (28) using Lipofectin (BRL) per the supplier's protocol or the calcium phosphate-mediated method of Graham and van der Eb (29)). Mock, pcDNA3/CAT, and PSMA3 transfections were done in parallel, and cells were harvested 48 hr after transfection for enzymatic assays.

RESULTS

Immunoscreening with Anti-NAALADase Antisera. A screen of ≈2 million plaques of a rat brain cDNA library in λgt11 yielded six immunopositive clones of apparently similar size. Sequences of the 3′ and 5′ ends of these six cDNAs (≈2500 bases) were identical. DNA sequence analysis of one of these clones (We) revealed a 3428-bp insert that contained an open reading frame of 1407 bases. The absence of an initiation codon indicated that this cDNA did not represent a complete coding sequence. To examine size and distribution of the mRNA species represented by the We clone relative to NAALADase activity, we did Northern analyses of rat brain, kidney, and liver RNA using the We cDNA as a probe. Fig. 1 shows that the distribution of detectable transcripts corresponded to the tissue expression of NAALADase activity with brain and kidney positive and liver negative. Interestingly, at least six transcripts were detectable in brain and kidney at estimated sizes of 5600, 5000, 2800, 2100, 750, and 500 nt. This is a larger population of transcripts than would be accounted for by the NAALADase activities previously described in brain and kidney homogenates by kinetic (13) or physical analyses (20), which show one or two compartments of activity, respectively, or by immunoblots of brain that show three specific immunoreactive bands (R.E.C., unpublished data).

Comparison of the sequence of the We clone to the GenBank sequence data bank revealed that bases 3–1428 of this rat brain cDNA were 86% identical to bases 1106–2534 of a cDNA previously isolated from the human prostatic carcinoma cell line LNCaP (27). This identity occurs mainly in the 3′ end of the coding region of the PSM cDNA and extends 23 bases into the 3′ untranslated sequence. The function of the protein described by this cDNA was unknown; it had been identified as a potentially useful clinical marker for prostatic carcinoma and was designated PSM (27, 30).

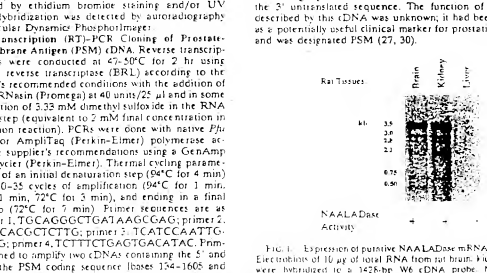

FIG. 1. Expression of putative NAALADase mRNAs in rat tissues. Electrophoresis of 10 μg of total RNA from rat brain, kidney, and liver were hybridized to a 1428-bp We cDNA probe. Note that the distribution of detectable transcripts correlates with the expression of NAALADase activity. We observe six transcripts of 5.5, 5.0, 2.8, 2.1, 0.75, and 0.5 kb in length, which are variably expressed in brain and kidney.

Coexpression of a Homologous mRNA and NAALADase Activity in Prostatic Carcinoma Cell Lines. To explore the possibility that the previously described PSM cDNA species encoded NAALADase, the LNCaP cell line from which it was cloned was assayed for NAALADase activity. Two prostatic carcinoma cell lines (DU145 and PC3) which had been reported to express neither the corresponding mRNA nor PSM antigen were assayed in parallel as negative controls. The PSM-positive LNCaP cell line exhibited high NAAG-hydrolytic activity, whereas the DU145 and PC3 (PSM-antigen-negative) lines were inactive (Table 1). Further examination of enzymatic activity in the LNCaP line revealed both high affinity and high-specific activity for NAAG (K_m = 0.5 ± 10 nM; V_{max} = 1215 ± 40 pmol/mg of protein per min). In addition, the observed NAAG hydrolysis was inhibited by quisqualic acid, a NAALADase inhibitor. These data indicated that the LNCaP peptidase activity was NAALADase. Northern analysis of the three prostate carcinoma cell lines was subsequently conducted and, consistent with its encoding a NAALADase protein, the W6 cDNA probe detected three RNA species from the LNCaP cell line and none in the DU145 or PC3 samples (Fig. 2). The predominant RNA species expressed by LNCaP cells (2.8 kb) is the same size as the PSM antigen transcript that was detected by the PSM cDNA clone of Israeli *et al.* (27).

Expression of PSM cDNA in NAALADase-Negative Prostate Carcinoma Cell Lines Confers NAAG-Hydrolyzing Activity. With evidence that the PSM antigen cDNA might encode NAALADase, RT-PCR was used to construct a 2436-bp cDNA (PSMA2) containing the putative 2250-base PSM open reading frame [as the original reagent (27) was unavailable out to patent application]. Cells not expressing NAALADase were transiently transfected with the PSMA2 DNA and assayed for NAALADase activity. As shown in Fig. 3, the PC3 line was successfully transfected with both calcium phosphate-mediated and liposome-mediated methods and demonstrated quisqualic acid-sensitive NAAG hydrolysis. The DU145 line also demonstrated quisqualic acid-sensitive NAAG hydrolysis after transfection with the calcium phosphate-mediated method. In addition, the observed NAAG-hydrolyzing activity was sensitive to the competitive NAALADase-inhibitor β-NAAG, providing further evidence for its similarity to the activity that had originally been characterized in rat brain. Mock-transfected and control (pcDNA3-chloramphenicol acetyltransferase)-transfected cells were NAALADase-negative, ruling out induction of the enzyme by the transfection procedure.

Sequence Analysis of the PSM cDNA with Regard to NAALADase Activity. After it had been determined that the PSM cDNA encoded a NAALADase, the cDNA and predicted amino acid sequences were examined for sequence similarities and structural characteristics that might be informative about the enzymatic activity of PSM. As had been reported by Israeli *et al.* (27), PSM appears to be a type II integral membrane protein, with a hydrophobic stretch of amino acids at positions 20–45 consisting of a putative membrane-spanning domain and three arginine residues at positions 16, 17, and 19 putatively serving as a basic cytosolic anchor. The predicted molecular mass of the PSM polypeptide is 84,000 Da, similar to the size of the deglycosylated rat brain NAALADase as determined by Western analysis after pretreatment of brain homogenates with endoglycosidase F (R.E.C., unpublished data). Further, potential sites of N-linked glycosylation occur

Table 1. NAALADase activity in prostatic carcinoma cell lines

Sample	[^{3}H]Glu, specific cpm	200 μM quisqualic acid
LNCaP	34,005 ± 185*	1256 ± 10†
PC3	28 ± 6‡	−20 ± 2§
DU145	53 ± 7‡	−30 ± 5§

Activity is expressed as specific cpm of [^{3}H]glutamate ([^{3}H]Glu) generated from [^{3}H]NAAG in 200 μl (1/10 vol) of assay reaction after subtraction of protein-free blank.

*Significantly different from protein-free blank by Student's *t* test ($P < 0.001$).

†Significantly different from LNCaP lysate alone by Student's *t* test ($P < 0.001$).

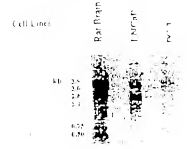

FIG. 2. Expression of putative NAALADase mRNAs in human prostatic carcinoma cell lines. Electrophoresis of 15 μg of total RNA from rat brain and the LNCaP, PC3, and DU145 cell lines were hybridized to a W6 cDNA probe. Among the cell lines, the distribution of detectable transcripts is limited to the NAALADase-positive LNCaP, in which 3.5-, 2.8-, and 2.3-kb species are seen.

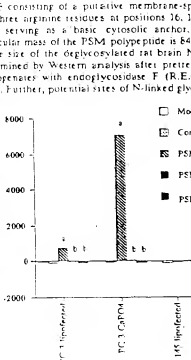

FIG. 3. NAALADase activity in PSM-transfected cell lines. One-hundred-microliter samples of PSMA2-transfected (PSM), pcDNA3-transfected (control), or mock-transfected (mock) cell lysates were assayed for NAALADase activity. Activity is expressed as described in Table 1. Error (SEM) is contained within the outlines of the bars in the graph. NAALADase inhibitors were included at a concentration of 200 μM (≈ 50 times their IC_{50} values against rat brain NAALADase (15, 25)). Data within each group were compared by a ANOVA with pairwise comparisons. Mock-transfected and control-transfected samples were devoid of NAAG-hydrolyzing activity relative to protein-free blanks (data not shown). a, Significantly greater than mock-transfected and control-transfected ($P < 0.001$ for all comparisons); b, significantly lower than respective PSM-transfected ($P < 0.001$ for all comparisons).

at nine positions in the putative extracellular domain of the protein. With regard to potential mechanisms for regulating NAALADase activity via posttranslational modification, a protein kinase C consensus site (Ser-321, Arg-323, Thr-326) within the short putative intracellular domain. With respect to the hydrolytic mechanism of NAALADase, the predicted amino acid PSM sequence was compared with those of other peptidases, but no functionally relevant similarities were found (22, 24).

DISCUSSION

PSM Is a NAALADase Enzyme. The search for NAAG-hydrolyzing enzymes in the rat brain led to the discovery and characterization of the extracellular acid-sensitive, high-affinity and high specific activity enzyme termed NAALADase (12, 24). As the transient transfection studies show, PSM exhibits important enzymatic characteristics of NAALADase as defined by the criterion of extracellular acid-sensitive NAAG hydrolysis. Thus, our interest in NAALADase in the nervous system has led us to discover a previously uncharacterized biochemical activity of this prostate cancer marker. Interestingly, a number of hydrolases have been identified as cancer marker antigens, including another prostate cancer marker, the prostate-specific antigen, which is a serine protease (25), and another brain peptidase, enkephalinase A, which was identified independently as the common acute lymphoblastic leukemia antigen (CALLA) (36, 37).

PSM was originally identified because its expression appeared to be restricted almost exclusively to prostatic epithelial cells in human tissues as determined by immunohistochemistry with the monoclonal antibody 7E11-C5 in human tissues (30). The antigen is expressed in both normal and neoplastic prostatic cells and in prostatic tumor metastases. Whereas other marker antigens for prostatic carcinoma such as prostatic acid phosphatase and the prostate-specific antigen are secreted proteins, PSM is an integral membrane protein. Thus, it is currently under investigation as a target for imaging and as a receptor for cytotoxic targeting modalities (38, 39). RT-PCR assays for PSM have also been examined for potential utility in the detection of hematogenous micrometastatic prostate cells (40).

The identification of PSM as NAALADase was originally surprising to us, given the original description of the highly restricted cellular expression of PSM (30). Later reports, however, revealed that low immunoreactivity levels to the 7E11-C5 antibody and its derivative CYT-356 (37, 38) and/or mRNAs detected by RNase protection assays using PSM-derived probes (41) were found in nonprostatic human tissues, including brain. A significant discrepancy nonetheless remains between the restricted pattern of expression of PSM in human tissues as detected by the aforementioned methods and the distribution of NAALADase in rat tissues including brain, kidney, sexual organs and peripheral nerves as determined by radioenzymatic assay (12, 14, 20, 22), and immunocytochemistry with anti-NAALADase antisera (20–22, 42). The poor correspondences between the distribution of PSM and rat NAALADase may reflect the existence of multiple NAALADase isoforms, some of which are not reactive to the available detection reagents for human PSM. Alternatively, there may be a species-related difference in NAALADase expression.

The identification of the NAALADase activity of PSM has potential impact on the study of its role in prostate biology and prostatic neoplasms. Given the localization of this enzyme to glandular epithelial cells, PSM may play a role in the local hydrolysis of peptides in prostatic fluid. Further, PSM may be responsible for the generation of extracellular glutamate in the compartment, as glutamate is known to be present in seminal fluid (43). Whether NAAG or other potential substrates for the enzyme may serve as glutamate precursors in this system remains to be determined. Additionally, the marked increase in the expression of PSM in malignant prostatic tumors raises the question of whether this cell-surface peptidase may play a role in the transformation of prostatic epithelial cells or in their ability to metastasize.

NAALADase: A Membrane Neuropeptide-Catabolizing Hydrolase. The identification of PSM as a NAALADase enzyme makes its corresponding cDNA the only NAALADase clone to have been characterized. The unique pharmacologic and tissue distribution of NAALADase indicate that it is a novel member of the membrane hydrolase family (12). The hydrolysis of NAAG by NAALADase in brain membranes is inhibited at least 80% by the general metalloprotease inhibitor orthophenanthroline and divalent metal chelators EGTA and EDTA. Conversely, its activity is increased by supplementation of divalent metal cations (12, 20, 44). Further, NAALADase is not inhibited by general serine or activated carboxyl protease inhibitors or by thiol-modifying reagents (12). These findings led us to the hypothesis that NAALADase was a metallopeptidase. Given that we are unable to identify significant sequence similarities between NAALADase and metallopeptidases that have been previously cloned and sequenced (34), however, a definitive mechanistic categorization of the enzyme will require further study.

Analysis of the translated PSM cDNA sequence yields information about the structural elements of one form of the NAALADase enzyme. The predicted general structure composed of a short intracellular domain, a single transmembrane element, and a large globular extracellular domain is common among membrane hydrolases (45). Multiple potential N-linked glycosylation sites account for the native antigen's high carbohydrate content. Also, the protein kinase C consensus site at Thr-16 identifies one possible mechanism for the regulation of NAALADase activity. Interestingly, the arachidonic acid metabolite 12(S)-hydroxyeicosatetraenoic acid has been found to enhance the motility and invasiveness of rat prostate AT2.1 tumor cells via activation of protein kinase C α (46). When considered together with our results, this raises the possibility that the regulation of the enzymatic activity of PSM by protein kinase C might increase the metastatic potential of prostatic tumors. As reported by Israeli *et al.* (27), the region of the PSM cDNA from nt 1531–1961 (as denoted in the GenBank sequence) is 54% identical at the nucleic acid level to the human transferrin receptor cDNA. It is unlikely that transferrin is a required component of the active NAALADase holoenzyme, however, because NAALADase activity appears to be represented by a single protein band in both nondenaturing and denaturing polyacrylamide electrophoresis gels (20).

Synaptic hydrolases play a critical role in modifying the action of signaling molecules in the nervous system. For example, ATP released from synaptic vesicles may either activate members of the P class of purinergic receptors or may be hydrolyzed locally to adenosine, which activates purinergic receptors of the A type (47). By analogy, we envision a similar role for NAALADase in the disposition of NAAG in which the intact dipeptide could bind to NAAG-sensitive receptors or, through the action of NAALADase, NAAG could serve as a source of synaptic glutamate. Whether intact NAAG or glutamate derived from NAAG would be the active species at a given synapse would depend on the type(s) of receptors present and the expression of NAALADase activity therein. In such a dual model, the regulation of NAALADase activity could be the fulcrum determining whether NAAG- or glutamate-mediated activity would predominate after NAAG release. In addition to serving this proposed function at neuronal synapses in the central nervous system, regulation of NAAG- and/or glutamate-mediated signaling by NAALADase may extend to other tissues where it is known to be expressed, such as in the neuromuscular junction (42) or the prostate gland (30).

implications for future studies of nervous system and psychiatric illnesses. Identification of an excitatory activity for PSM will facilitate the characterization of its role(s) in normal and malignant prostate cell function and further address its utility as a prostate cancer marker and targeting functionality. The discovery of a NAALADase-encoding cDNA has resulted in the identification and beginning of the molecular characterization of what appears to be a family of NAALADase-like species. Additionally, the cDNA provides a genetic tool for studying NAALADase expression at the transcriptional level, which will be particularly useful in determining its cellular distribution in the complex networks of closely apposed cells in nervous tissue.

In the broader context of excitatory neurotransmission, the characterization of NAALADase is a crucial step in understanding the disposition of NAAG and the role this neuropeptide may play in glutamatergic signaling. Moreover, the observation that overregulation of glutamate, NAAG, and NAALADase/PSM occurs in a variety of human disorders is substantial evidence that further study of NAALADase may have important clinical applications both within and beyond the nervous system.

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